FINAL REPORT

ON

CONTRACT NO. NAS 9-8264

Contractor: University of Houston
Houston, Texas

DEVELOPMENT OF LUNAR RECEIVING LABORATORY

BIOLOGICAL PROTOCOL

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FINAL REPORT
ON
DEVELOPMENT OF LUNAR RECEIVING LAB.
BIOLOGICAL PROTOCOL

REF N.A.S.A., # NAS 9-8264

This is the final report of the work achieved on the N.A.S.A. Contract NAS 9-8264 entitled "Development of Lunar Receiving Laboratory Biological Protocol." This Contract was originally started for the period of July 1, 1968 - June 30, 1969. Since then, the contract was extended from July 1, 1969 - October 31, 1969 and further extended to August 31, 1970. The title of the Contract remained unchanged.

The original contract from July 1, 1968, was to develop the Botany program as part of the Lunar Receiving Laboratory Biological Protocol. This program included selection of specific algae, seeds, and seedlings of higher plants and to establish tissue cultures of plants of economic importance.

As of November, 1968, the contractor was advised that his primary responsibility to this contract would be to furnish 40-50 tissue culture media of each of 8 representative plants and the same number of initial tissues of each of the 8 species.

This final report consists of Parts I, II, III, IV and Appendix I and II.

PART I

DUTIES PERFORMED BY THE CONTRACTOR

Efforts were directed to establish the best culture media and to maintain excellent growth of the tissue cultures of selected representative lower and higher plants for Apollo 11 and 12. The final culture media used for each tissue(s) is indicated in appendix.

All the culture media were established on the basis of previous literature, reference and nutritional requirements of each species. The tissue cultures required very frequent transfers under total asceptic conditions so that enough materials would be available for test systems during the Apollo missions. All efforts during this work period were directed toward this objective. Enough stock materials of these 8 test systems were maintained at the University of Houston and the Contractor provided culture media and the tissues of the various plant systems.

In the following pages, details of these shipments to the Lunar Receiving Laboratory are presented: the Contractor provided all the botanical test systems and media as required by the Contract for the Apollo 11, 12 and 13 test missions. These were delivered to the Lunar Receiving Laboratory as and when they were needed.

April 25, 1969	Delivered 80 plant tissue cultures of 8 genera, 10 mother cultures/each genus and 400 bottles of culture media = 1st. simulation.
May 23, 1969	Delivered 80 plant tissue cultures of 8 genera 10 mother cultures/each genus and 400 bottles of culture media = 2nd. simulation.
June 27, 1969	Delivered 80 plant tissue cultures of 8 genera, 10 mother cultures/each genus and 400 bottles of culture media.
July, 1969	Delivered 10 mother cultures/each of the 8 plants and 400 bottles of culture media. This material was to be tested with the lunar material after the Apollo 11 mission.
July, 1969	Delivered 10 mother cultures/each of 4 plants and 200 bottles of culture media. This was to be used for Apollo 11 also.
August 1, 1969	Delivered 10 mother cultures of tobacco, 10 mother cultures of soybean and 50 bottles of tobacco culture media and 50 bottles of soybean media for subcultures of materials to be tested with lunar soil after the Apollo 11 mission.
August 8, 1969	Furnished Pinus lambertiana (sugar Pine) and Pinus palustris (Longleaf). 10 mother cultures of both of the species were delivered. 100 bottles of Pinus culture media were also delivered.

The following materials were supplied to the Lunar Receiving Laboratory at NASA on these dates for the month of November.

November 3, 1969

HABITUATED TOBACCO	4 mother cultures for 20 cultures
PINE	4 mother cultures for 20 cultures
RICE	5 mother cultures for 25 cultures
CORN	4 mother cultures for 20 cultures
WHITE'S IOX MEDIUM for Tobacco	25 bottles
MUK MOD. MEDIUM for Pine	25 bottles
HELLER'S MOD. MEDIUM for Rice	25 bottles
WHITE'S IX MEDIUM for Corn	20 bottles
HOAGLAND'S MEDIUM for Marchantia	25 bottles

November 12, 1969

1/2 Roll polypropylene

November 13, 1969

LYCOPODIUM sp.	40 cultures
TODEA BARBARA	40 cultures

November 21, 1969

White's IX Media	56 bottles
Heller's Media	56 bottles
S.V. Media	56 bottles

Cultures

Corn	11 mother cultures	(30 cultures)
Euphorbia	1 culture on Muk. IAA Kin	(3 cultures)
Pine (Pinus palustrus)	14 cultures for	(40 cultures)
Sunflower	10 mother cultures	(40 cultures)

November 26, 1969

56 bottles of Modified Muk. Medium

8 mother cultures of Albino Tobacco--for 24 sub-cultures.

The following materials were supplied to the Lunar Receiving Laboratory at N.A.S.A. on these dates:

December 29, 1969

S.V. Media	150 bottles
MUK. MOD. MEDIUM for Pine	50 Bottles
WHITE'S IX MEDIUM for Corn	50 bottles
WHITE'S 10X MEDIUM for Tobacco	50 bottles

January 5, 1970

S.V. Media	150 bottles
WHITE'S 10X MEDIUM for Tobacco	50 bottles
WHITE'S 1X MEDIUM for Corn	50 bottles
HELLER'S MOD. MEDIUM for Rice	50 bottles
HOAGLAND'S MEDIUM for Marchantia	50 bottles
MUK. MOD. MEDIUM for Pine	

February 4, 1970

Assorted culture of Haplopappas.

Habituated, Soy, Sunflower 300 cultures

February 21, 1970

MARCHANTIA mother culture	20 bottles
LYCOPODIUM sp.	20 bottles
TODEA BARBARA	20 bottles
HOAGLAND'S MEDIUM for Marchantia	50 bottles
KNUDSON'S MEDIUM	100 bottles

March 4, 1970

Written description of tissue cultures.

March 20, 1970

S.V. Media	200 bottles
HELLER'S MOD. MEDIUM for Rice	50 bottles
HOAGLAND'S MEDIUM for Marchantia	50 bottles
WHITE'S 10X MEDIUM for Tobacco	50 bottles
WHITE'S 1X MEDIUM for Corn	50 bottles
MUK MOD. MEDIUM for Pine	150 bottles
MUK + IAA + KINETIN	50 bottles

Cultures:	Mother Cultures
Euphorbia	10 (for 40 cultures)
Pine (Pinus palustrus)	18 (for 40 cultures)
Pinus elliotus	8 (for 40 cultures)
Pinus Lambertiana	10 (for 40 cultures)
Haplopappus	18 (for 40 cultures)
Habituated tobacco	18 (for 40 cultures)
Soybean	18 (for 40 cultures)
Sunflower	18 (for 40 cultures)
Marchantia	18 (for 40 cultures)
Rice	18 (for 40 cultures)
Corn	18 (for 40 cultures)
Carrot	4 (for 40 cultures)

March 22, 1970

MUK MOD. MEDIUM	150 bottles

March 23, 1970

S.V. Media and ascorbic acid	100 bottles

March 24, 1970

S.V. Media	100 bottles

April 2nd., 1970 Cultures were supplied as follows:

Carrot cultures	(3)	to give 10-12 cultures
Sunflower	(14)	to give 56+ cultures
Pinus palustris	(40)	to give 40-80 cultures
Habituated tobacco	(11)	to give 44+ cultures
Haplopappus	(12)	to give 48+ cultures
Soy bean	(12)	to give 60+ cultures

April 15, 1970 Media was supplied as follows:

S.V. + Asc.	150 bottles	for Haplopappus, Soy, Sun.
MUK MOD.	150 bottles	Pine (3 species)
MUK + IAA + KIN	50 bottles	Euphorbia
HOAGLAND'S	50 bottles	Marchantia
KNUDSON'S	100 bottles	Ferns (2 species)
WHITE'S 1X.	50 bottles	Corn
WHITE'S 10X	50 bottles	Habituated tobacco
S.V. + IAA + KIN	50 bottles	Carrot
HELLER'S	50 bottles	Rice
WHITE'S 1x + NAA + 2,4D	50 bottles	

Cultures were supplied as follows:

Marchantia	(14)	to give 42 cultures
Pteridium aqualinium	(24)	to give 48 cultures

April 21, 1970

Written preliminary report. "Preparation Media."

April 22, 1970

WHITE'S 10X	50 bottles
S.V.	75 bottles
HELLER'S	30 bottles
MOD. MUK.	50 bottles

May 23, 1970

WHITE'S 10X	50 bottles
MOD. MUK.	50 bottles

May 25, 1970

S.V. Media	75 bottles
HELLER'S	30 bottles

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CAPITAL EQUIPMENTS

The following capital equipments were purchased on this contract;

1. PR-6 International Centrifuge with Head # 209
2. Agnew-Higgins Model 43 Transfer Hood
3. Distilling Apparatus
4. Sorvall RC-2 Ultracentrifuge with rotor
5. 2 gyro rotators shakers.
6. CO-2 incubator (in part purchased from funds of separate budget.

CONSULTANTS

During the period of this Contract, a number of consultants either participated in work at the University of Houston or visited.

Dr. James E. Mann, Assistant Professor, University of Houston carried out preliminary work on refining the algal system for six weeks during the summer of 1968.

Dr. Peter K. Chen, Associate Professor, Georgetown University Washington D.C. was a visiting consultant for four weeks during the summer of 1969. He carried out work on the effect of basalt on tobacco tissue cultures.

The following consultants visited the University of Houston and the Lunar Receiving Laboratory for discussion. They also provided plant materials as test systems for Apollo 11, 12 and 13. They were:

1.	Dr. Gus DeMaggio Professor of Biology Dartmouth College Hanover, New Hampshire	1. Provided initial materials, of Pteridium and Todea. 2. Visited the site to interpret the results of Apollo 11 experiments
2.	Dr. Claude Brown Professor of Biology University of Georgia Athens, Georgia	1. Provided 3 species of Pine tissue cultures. 2. Discussed the maintenance of these cultures and composition of media.
3.	Dr. Howard Arnott Professor of Botany University of Texas Austin, Texas	1. Discussed the interpretation of light and electron micrographs of the test systems.
4.	Dr. Carl R. Partanen Professor and Chairman Department of Biological Sciences University of Pittsburgh Pittsburgh, Pennsylvania	a. Provided Pteridium cultures for Apollo 13. b. Considered a fern expert and noted cytologist in this country. c. Was a consultant in designing the original Botany protocol in 1967.
5.	Dr. Ian M. Sussex Osborn Botanical Laboratory Yale University New Haven, Connecticut	a. Provided carrot cultures for Apollo 13. b. Considered a lower plant and tissue culture expert. c. Was a consultant in designing the original Botany protocol in 1967.

ACKNOWLEDGEMENT is given to the following people who contributed to the successful performance of the task related to this Contract NAS 9-8264 at the University of Houston.

Dr. K. Raghu

Dr. J. W. Weete

Dr. J. E. Mann

Miss Kathy Lucas

Mrs. Kathy Yoeng

Mr. Wade Bolton

Miss Juliana Wong

Miss Debbie Mutchler

PART II

I. Algal Cultures

DATA ON DUTIES PERFORMED AT THE UNIVERSITY OF HOUSTON WITH CONCURRENT COLLABORATION WITH THE LUNAR RECEIVING LABORATORY DURING CONTRACT PERIOD (JULY, 1, 1968 - AUGUST 30, 1970) IN ORDER TO HAVE BASE-LINE DATA.

1. ALGAL CULTURES

Established algal cultures of 9 different species of algae were sub-cultured with 20-25 tubes once every 3-4 weeks. These algae were:

Chlorella pyrenoidosa

Anacystis nidulans

Phaeodactylon tricormutum

Porphyridium cruentum

The following procedures were adopted to prepare media, transfer and maintain algal cultures. These above cultures were maintained in appropriate media as shaking cultures or as cultures maintained with a O_2-CO_2 manifold. Details of these media are included in the following pages.

After the beginning of this contract (July 1, 1968), it was decided to have only 4 representative species of major groups of algal cultures. They are:

1. Anacystis
2. Chlorella
3. Phaeodactylon
4. Porphyridium

PREPARATION:

1. Prepare medium according to the requirement of the species.
2. For preparation of 20-40 slants, add 3 grams of agar to 200 ml of liquid medium.
3. Heat to boiling in beaker, while stirring on magnestic, until agar dissolves completely.
4. Dispense prepared agar in 5 ml portions into sterile, plugged 15 ml tubes.
5. Autoclave for 15 minutes.
6. Lean hot sterile tubes against board on clean flat surface at about a 30° angle until the agar is solid. Store upright under aseptic conditions.

Transfer of Maintenance:

1. Working in transfer chamber, remove the cotton plug from the stock slant and flame top two inches of tube over burner.
2. Flame wire of inoculating needle in the tip of flame and allow to cool.
3. Insert inoculating needle in tube, draw out small amount of inoculum.
4. Replace plug in tube.
5. Use fresh sterile slant, remove plug, and flame top of tube.
6. Insert needle with inoculum, and draw loop up slant against agar in a wavy motion.
7. Replace plug in transfer slant.

These cultures were maintained under 200 to 300 foot candles of light for approximately 3 weeks at 65° to 75°F.

II. SEED GERMINATION:

Seed germination was carried out on Brassica oleracea var. Greenback, Lycopersicon var. Homestead, and Hordeum vulgare var. Moore. The criteria of germination was when the seed coats opened and the young embryos came out of the cotyledon. The percent germination was recorded daily for 5 petri dishes/each 25 seeds. The Oat seeds were dehusked in this experiment, since germination was a factor on those seeds that were not dehusked.

Medium Seeds were generally germinated on petri plates containing filter paper soaked in distilled water or a Knop's solution. This medium varied with different species.

Seed germination of the above species was also carried out with the addition of Basaltic materials to simulate Lunar soil. The procedure was:

1. Sterilize seeds for ten minutes in a 10% chlorox solution (dehusking the oat seeds before sterilization)
2. Using sterile forceps, place 25 of each specie into 5 petri dishes on filter paper. (All equipment and procedures under germ-free conditions).
3. Disperse 5 grams of autoclaved Basalt soil evenly into the petri dishes.

The following data were obtained for an 8-day period.

RESULTS:

Control: # of Seeds Germinated

	Days								
Plates	1	2	3	4	5	6	7	8	
I	12	88	100	100	100	100	100	100	C
II	28	84	92	96	96	96	96	96	A
III	16	60	88	92	96	96	96	96	B
IV	48	96	100	100	100	100	100	100	B
V	16	80	92	96	96	96	96	96	A
Mean $\bar{N}$ =	24	82	94	97	97	97	97	97	G E
I	0	0	68	100	100	100	100	100	T
II	0	0	92	96	100	100	100	100	O
III	0	0	88	96	96	96	96	96	M
IV	0	0	92	96	100	100	100	100	A
V	0	0	96	100	100	100	100	100	T
Mean $\bar{N}$ =	0	0	87	98	99	99	99	99	O
I	36	36	72	84	84	84	84	84	O
II	44	48	72	92	96	96	96	96	A
III	52	64	76	88	88	88	88	88	T
IV	24	28	72	92	92	92	92	92	
V	16	32	72	84	96	96	96	96	
Mean $\bar{N}$ =	34	42	73	88	91	91	91	91	

Basalt Added: Percent Germination

Plates	Days 1	2	3	4	5	6	7	8	
I	8	72	88	88	88	88	88	88	C
II	20	96	100	100	100	100	100	100	A
III	28	84	88	96	96	96	96	96	B
IV	44	84	84	92	92	92	92	92	B
V	12	88	92	92	92	92	92	92	A
Mean $\bar{N}$ =	22	85	90	94	94	94	94	94	G E
I	0	0	60	96	100	100	100	100	T
II	0	0	36	80	100	100	100	100	O
III	0	0	80	100	100	100	100	100	M
IV	0	0	60	96	100	100	100	100	A
V	0	1	56	80	96	96	96	96	T O
Mean $\bar{N}$ =	0	0	58	90	99	99	99	99	
I	36	48	88	88	88	88	88	88	O
II	20	56	76	80	84	84	84	84	A
III	40	68	80	100	100	100	100	100	T
IV	40	44	72	80	88	88	88	88	
V	36	68	84	88	88	88	88	88	
Mean $\bar{N}$ =	34	57	80	87	89	89	89	89	

From the above data it can be concluded that the addition of basalt to the germinating seeds, had no observable effect.

III. TISSUE CULTURE

Already established tissue cultures of the following 8 different plant materials were subcultured every 3-4 weeks. They were:

1. *Nicotiana tabaccum* (Tobacco)
2. *Glycine soja* (Soybean)
3. *Helianthus annuus* (Sunflower)
4. *Zea mays* (Corn)
5. *Oryza sativa* (Rice)
6. *Pinus palustrus* (Long leaf Pine)
7. *Pinus lambertiana* (Sugar Pine)
8. *Marchantia polymorpha* (Liverwort)

The last material, viz. *Marchantia polymorpha* is a Bryophyte classified under lower plants. This material was introduced as a test system (November, 1968) and special efforts were directed to establish them under aseptic conditions.

Gemmae of *Marchantia polymorpha** (Liverwort, gametophyte) were initiated on mineral agar from gemae. The gemmae were removed from gemmae cups by placing a drop of commercial 3% hydrogen peroxide in each cup. The hydrogen peroxide loosens the gemmae and causes them to float to the surface of the drop. With the aid of a sterilized artist's brush, gemmae are transferred from the cup to a test tube containing sterile distilled water. Standard aseptic techniques were employed in the subsequent procedures, which are accomplished in a transfer chamber equipped with a germicidal lamp.

Gemmae were removed from the water suspension by filtration through filter paper and were washed with eight aliquots of dilute sodium hypochlorite solution prepared by mixing 1 volume of commercial 5.25% solution (chlorox) with 30 volumes of sterile

distilled water. The filter paper with adherent gemmae were removed from the funnel, placed in a petri plate, and moistened with several drops of water. With a dissecting needle, the gemmae were transferred to sterile media in petri plates on Hoagland's media

Acknowledgements are made to Dr. Morton Miller, Assistant Director, and to Dr. Paul Voth, University of Chicago for providing fresh cultures of Marchantia polymorpha and their advice and assistance.

Two experiments were carried out on tobacco tissue cultures adding basalt on the media. These experiments are described as Experiment I and II in the following pages.

EXPERIMENT I

During the period of this Contract an experiment was set up to test the growth of tobacco plant tissue cultures on media containing terrestrial basalt. Details are described below.

Title: Growth of Tobacco Plant Tissue Culture in Media Containing Terrestrial Basalts.

Abstract: An Experiment was designed to determine whether tobacco tissue cultures can be grown in the presence of terrestrial basalts added to the culture medium.

Plan of Work: Samples of terrestrial basalt obtained from Texas Coast and provided by the Geology Department were tested. When the basalts were added to the culture media they were suspended in the liquid phase and finally settled down at the bottom of the agar medium. Therefore they were added to the top surface of the solid agar medium (White 1963). 10 X concentration of the basic salts of White's media was used as the final composition of the culture medium. This medium (20 ml/bottle) was employed because the contractor found it satisfactory for rapid growth of tobacco plant cells (Venketeswaran, 1968). Agar 1% was used for the solid media. Masses of cells which were at their log phase of growth were used as inocula. By using this method, a relatively homogeneous population of cells were obtained. A minimum of ten replicates were employed in each of the 3 concentrations of basalt (.2 gms, .6 gm and 2 gms/sample) and the control. Analyses of growth was made obtaining fresh weight (F.W.) and dry weight (D.W.) at the weekly intervals.

Conclusion: As can be observed, no significant difference was obtained between the control and the low concentration of basalt employed.

One possible explanation is because the basalt was added to the surface of the culture medium and the tissue culture was inoculated on the top of this basalt. This could have prevented or affected the transport of nutrient ions to the cells for their optimum growth. Therefore, at present, experiments are designed to sprinkle or dust terrestrial basalts on the top of the cultures and observe their effects on growth of these cultures. These will be reported in the following communication.

Reference

Venketeswaran, S. (1968). Final report of N.A.S.A. Contract 9-6822.

White, P. R. (1963). The Cultivation of Plant and Animal Cells. Ronald Press Co., New York.

FRESH AND DRY WEIGHT OF TOBACCO TISSUE CULTURES GROWN IN DIFFERENT BASALT CONCENTRATIONS

Week	Weight	A Control	B 1% .2 gms	C 3% .6 gms	D 10% 3 gms
1	Fresh	3.0013	1.95839	2.43531	2.87955
	Dry	0.31545	0.10313	0.20081	0.20949
2	Fresh	9.5937	5.77602	5.74191	3.85378
	D.W.	0.49054	0.3817	0.34292	0.29786
3	F.W.	12.25447	10.55834	10.03694	6.5913
	D.W.	0.48553	0.42212	0.4054	0.34460
4	F.W.	13.35384	11.87815	10.705.6	8.32826
	D.W.	0.53418	0.48843	0.47505	0.44375

EXPERIMENT II

An experiment concerning the effect of Basaltic soil on the growth of tissue culture was carried out. Earlier fresh weights were obtained on one week intervals for a total of 4 weeks. The basalt concentration was: (1) o percent, (2) 1%, (3) 3%, (4) 10%.

Procedure:

1. Prepared 4 liters of White's 10X solid media.
2. Added O (zero) basalt to the control, 10 grms for the 1%, 30 grams for the 3%, and 100 grams for the 10%.
3. The media was then poured into the containers (25 ml per jar) while continually swirling the media.
4. The media was then autoclaved and cooled. Tissue cultures of tobacco were transferred aseptically with initial weights being recorded. Fresh and dry weights taken at weekly intervals for 4 weeks and statistical analyses are given below.

Fresh Weight Initial: $\bar{N}$ = .71829

Average Fresh Weight (grams)

	Weeks			
	1	2	3	4
Control	2.0160	2.0302	3.0092	3.8633
1% Basalt	1.5.6.	1.6776	2.0789	2.6669
3% basalt	1.7447	1.5780	2.2097	2.1451
10% Basalt	1.2914	1.5161	2.0730	1.8902

Average Dry Weight (grams)

	Weeks			
	1	2	3	4
Control	0.1681	0.1235	0.1814	0.2377
1% Basalt	0.1124	0.1079	0.1364	0.1838
3% Basalt	0.1682	0.1080	0.1608	0.1254
10% Basalt	0.1529	0.1033	0.1481	0.1437

Fresh Weight

Control	Variance	Standard Deviation	Standard Error of the Mean
1st. week	0.2344	0.4842	0.1531
2nd. week	0.2499	0.4999	0.1581
3rd. week	0.3524	0.5936	0.1857
4th. week	1.7995	1.3415	0.4242
1% Basalt			0.0826
1st. week	0.0682	0.2612	0.1639
2nd. week	0.2687	0.5184	0.1375
3rd. week	0.1890	0.4347	0.2162
4th. week	0.4676	0.6838	
3% Basalt			
1st. week	0.0591	0.2432	0.0769
2nd. week	0.1553	0.3941	0.1246
3rd. week	0.2356	0.4853	0.1535
4th. week	0.1826	0.4273	0.1351
10% Basalt			
1st. week	0.0355	0.1883	0.596
2nd. week	0.0566	0.2378	0.0752
3rd. week	0.2424	0.4924	0.1557
4th. week	0.3416	0.5844	0.1848

PART III

DESCRIPTION OF TISSUE CULTURES SUPPLIED TO N.A.S.A.

The technical monitor (Dr. C. H. Walkinshaw) requested a description of the tissue cultures supplied to N.A.S.A. for the Apollo quarantine tests on the basis of their growth and development. Excellent, Good and Poor were considered on an arbitrary basis. Description of each of the tissues and their condition are described.

Marchantia polymorpha L.	Excellent:	The gemmae germinate within two to three days and the plants grow rapidly, putting out dense, white rhizoids and long green thali. The best growth is obtained under conditions of constant illumination.
	Good:	The thali turn brown with age and the growth is decreased. They usually produce gemmae which are of use to start new cultures. This browning can also be caused by excessive heat and insufficient light.
	Poor:	The gemmae are either slow growing or they turn brown and discontinue their growth. On older plants poor condition is demonstrated by browned and dry thali or by reddening of the thali.

Lycopodium cernuum L.	Excellent:	The plants are light green in color. They multiply fairly rapidly, covering the bottom of the jar within eight to nine weeks. They are very sensitive to heat and grow at a faster rate if they are kept under constant illumination.
	Good:	The tissue is green but the growth rate is very slow.
	Poor:	The tissues are either brown or white. This change in the color is caused most often by insufficient lighting and/or excessive temperatures. The tissues can seldom be recovered after they have reached this condition.

Todea barbara (L.) Moore	Excellent:	The plants are dark green in color. They multiply at a speed that covers the bottom of the jar within eight to nine weeks. These plants are sensitive to heat and must be kept from being too close to the lighting in the chambers to prevent them from being scorched.
	Good:	The tissue is similar to that described above except the growth rate is lower.
	Poor:	The tissues turn brown in color and seldom ever recover.

Pinus palustris Mill.	Excellent:	The tissue is pale green in color and moist. It is also friable. The growth rate is such that the tissue doubles every four weeks.
	Good:	The color is tan to brown and the growth rate is slow.
	Poor:	There is a minor difference between the good and poor tissues; the poor tissue is brown, drier and grows at a slower rate.

Pinus ellioti Engelm.	Excellent:	The tissue is yellow in color due to the high caretenoid content of the tissue. The tissue is friable, and moist. The growth rate is such that the amount of tissue doubles every four weeks.
	Good:	The tissue remains moist and friable but is light brown in color and the growth rate is slower.
	Poor:	The tissue is brown in color. It tends to be slightly dry and t the growth is extremely slow.

Pinus lambertiana	Excellent:	The tissue is green in color, is friable and moist. The growth rate is such that the tissue doubles in size about every four weeks.
	Good:	The tissue is green and brown in color. It is moist and friable, however, the rate of growth is decreased.
	Poor:	The tissue is brown, slightly dry and very slow growing.

Glycine soja (L) Sieb. & Zuck.	Excellent:	The tissue is green, friable and moist when grown on S.V. medium. The growth rate is such that there is doubling in size of the tissue every two weeks. The tissues should be undifferentiated.
	Good:	The tissue is dark green to brown in color. It is slow growing, undifferentiated, and friable.
	Poor:	The tissue is brown, hard and slow growing. It has been found to be sensitive t to light and temperature conditions. The optimal conditions being 72-78 degrees with constant illumination.

Helianthus annuus L.	Excellent:	The tissue is dark green, undifferentiated, moist and friable. The growth rate is such that the amount of tissue nearly triples over a three week period when grown on S.V. medium at 72-78 degrees Fahrenheight and under constant illumination.
	Good:	The tissue is light green to light brown in color, moist and friable. In some cases there is differentiation in the form of roots. The growth rate is somewhat slower than that of the excellent tissue. The above conditions often are the result of infrequent transfer of the tissue and or improper lighting or temperature controls. It has been found that the tissue is very temperature-sensitive.
	Poor:	Tissues are brown, very slow growing, and often excessively dry or wet.

Haplopappus gracilis (Nutt.) Gray	Excellent:	The tissue is light yellow to light green in color, forms a fairly firm, lumpy callus. The growth rate is relatively rapid, the tissue doubles in size within two or three weeks.
	Good:	The color of the tissue is dark brown and the growth rate is very slow.
	Poor:	There is little difference in the color of the poor tissue and the good tissue, however, the growth rate of this tissue is extremely slow and the calli are hard and dry.

Nicotiana tabacum L.	Excellent:	This tissue is very moist and friable, it is green and has a rapid rate of growth (approximately tripling in size in a period of two weeks).
	Good:	The color varies between yellow green and white. There is a slower rate of growth than is exhibited by an excellent tissue and often the tissue is much drier.
	Poor:	The tissue is often very dry. Has a low rate of growth and is brown in color.

Euphorbia terracena	Excellent:	The tissue when grown on MUK medium containing IAA and kinetin is dark green in color. It forms a firm tissue which doubles in size within two to three weeks.
	Good:	The tissue may have light brown regions among the green. The growth rate of the green portions remains fairly rapid. The browning is caused by the heat of the lights.
	Poor:	The tissues turn brown, become unusually moist and usually die soon after they have reached this condition.

Zea mays L.	Excellent:	This tissue in culture forms a firm, yellow callus. It is slow growing and tends to differentiate to form roots if care is not taken to prevent this. In this grade of culture there should be a minimum of differentiation.
	Good:	The cultures would appear as described above, however, there is more differentiated tissue.
	Poor:	This tissue is excessively differentiated and in some cases has turned brown.

Oryza sativa L.	Excellent:	The tissue is pale yellow, slightly dry and friable. It has an extremely slow rate of growth.
	Good:	There is really no intermediate between the excellent and the poor cultures.
	Poor:	The tissue is dry and brown. There is very little if any growth.

PART IV - COMMUNICATIONS

The Contractor presented the following presentations in the form of Abstracts at International and National meetings. He also co-authored a paper from which experimental procedures are employed in work related to Apollo 11, 12 and future programs. These presentations are as follows:

1. Venketeswaran, S., C. H. Walkinshaw, H. C. Sweet, and W. Jackson. University of Houston, U.S.D.A. (Forest Service), and NASA Lunar Receiving Laboratory; Brown and Root-Northrop, Houston, Texas; and Dartmouth College, Hanover, N. H. DEVELOPMENT OF THE BOTANICAL PROTOCOL FOR THE APOLLO MISSION. Amer. J. Bot. 57:736.
2. Venketeswaran, S., C. H. Walkinshaw, and H. C. Sweet. University of Houston; U.S.D.A. (Forest Service) and NASA Lunar Receiving Laboratory; and Brown and Root-Northrop, Houston, Texas. STUDIES ON TISSUE CULTURES OF HIGHER PLANTS AFTER EXPOSURE TO LUNAR SOIL AFTER APOLLO XI AND XII). Amer. J. Bot. 57:736.
3. Sweet, H. C., C. H. Walkinshaw, and S. Venketeswaran. Brown and Root-Northrop, U.S.D.A. (Forest Service), NASA Lunar Receiving Laboratory, and University of Houston, Houston, Texas. REACTIONS OF SEVERAL SPECIES OF LOWER PLANTS TO LUNAR MATERIAL). Amer. J. Bot. 57:736.
4. Weete, J. D., S.Venketeswaran, and J.L. Laseter. TWO POPULATIONS OF ALIPHATIC HYDROCARBONS OF TERATOMA AND HABITUATED TISSUE CULTURES OF TOBACCO (MS accepted).

Copies of these publications are submitted along with this report

RACINA.—Non-articulated laticifer initials become identifiable in the young embryo upon development of the cotyledons. Eight laticifer initials are formed in *E. terracina*. The initials arise at the level of the cotyledonary node and each is positioned along the lateral outermost edge of the four procambial strands which are present in the embryo. The number of initials formed appears to be related to the number of procambial strands that differentiate in the embryo, for in another species, *E. marginata*, the 12 initials are related in a similar way to the six procambial strands present in that species. The initials during subsequent development form branches which grow horizontally into the cortex and along the periphery of the procambial strands. As the embryo enlarges additional branches grow into the cotyledons; their growth along the hypocotyl toward the root meristem remains limited in this small embryo prior to germination.

11:30 **Vertrees, G. L.** Indiana University, Bloomington.—CALLOSE IN LACTICIFERS OF CICHORIUM INTYBUS.—Irregularly formed deposits of callose are demonstrated in the articulated laticifers of *C. intybus* following staining with anilin blue fluorochrome or tannic acid-iron chloride-lacmoid. These deposits, which are not specifically localized in relation to cell wall perforations but which sometimes occlude the lumen locally, are more frequently seen in older than in younger laticifers and may be especially numerous near severed ends. The possible role of laticifer callose as a plugging and sealing substance will be discussed.

11:45 **Sussex, Ian M., Mary E. Clutter,** and **Mary Helen Goldsmith.** Yale University, New Haven, Conn.—CELL DIFFERENTIATION AND AUXIN TRANSPORT.—After vascular tissue in the stem of tobacco is severed, redifferentiation of pith cells in the wound area occurs in two distinct stages. In an initial period that lasts more than seven days divisions in pith cells occur in random planes. Subsequently, division planes become restricted to the longitudinal axis and a wound cambium that consists of elongated cells is differentiated. On first wounding, auxin transport through the wound is reduced to about one third of intact stem values, and auxin accumulates in the stem above the wound. Transport increases during the period when cell divisions are occurring randomly, and by the time a wound cambium is differentiated throughout the wound, auxin transport rates are restored to values near those for the intact stem. The results imply that increasing auxin transport results in orientation of the plane of cell division and differentiation of elongated cells of the vascular tissue.

12:00 Luncheon and Business Meeting

1:30 Session 5. Tuesday Afternoon. Symposium.

FERNS AS TOOLS IN SOLVING BIOLOGICAL PROBLEMS. Joint meeting with the General Section and the American Fern Society.

Session 1. Wednesday Morning. DAVID A. STETLER, *presiding*.

9:00 **Venketeswaran, S., C. H. Walkinshaw, H. C. Sweet,** and **W. Jackson.** University of Houston, U.S.D.A. (Forest Service), and NASA Lunar Receiving Laboratory; Brown and Root-Northrop, Houston, Texas; and Dartmouth College, Hanover, N. H.—DEVELOPMENT OF THE BOTANICAL PROTOCOL FOR THE APOLLO MISSION.—This protocol was developed to design experiments and select botanical test systems in order to determine whether lunar material of Apollo mission can cause any effects on plant challenge systems grown under controlled asceptic conditions. The four challenge systems were: algae, spores and seeds, seedlings, and tissue cultures of Bryophytes, Pteridophytes, and higher plants. The justification and reason for selection of the different species were: crop plants, availability, pathogen-free, disease susceptibility, literature information, and uniqueness of the genera. Tissue cultures were selected particularly on the basis of availability, growth characteristics, and nutritional requirements. Details of the experimental design and procedures on the algal, spore and seed, seedlings, and tissue cultures will be described. Addition of contingent species to Apollo 12 on the basis of the results after Apollo 11, will be disclosed. Emphasis on development of germ-free plants, their seeds, etc., will be treated also. Preliminary results of the influence of lunar soil on plants and plant tissue culture after the Apollo 12 mission will be presented.

9:20 **Venketeswaran, S., C. H. Walkinshaw,** and **H. C. Sweet.** University of Houston; U.S.D.A. (Forest Service) and NASA Lunar Receiving Laboratory; and Brown and Root-Northrop, Houston, Texas.—STUDIES ON TISSUE CULTURES OF HIGHER PLANTS AFTER EXPOSURE TO LUNAR SOIL AFTER APOLLO XI AND XII.—Tissue cultures of a number of plants of economic importance (pine, corn, rice, soybean, sunflower, tobacco, *Haplopappus*, and *Euphorbia*) were employed to study the effects of the lunar soil from the Apollo mission on the growth, development, and morphogenesis. Details of the text procedures, etc. have been described in the Botanical Protocol of the Lunar Receiving Laboratory, Manned Spacecraft Center (July 1969, pp. 592–625). No deleterious or toxic effects of the lunar soil on growth and development of plant tissue cultures were observed. Certain plant tissue cultures, viz., Sunflower (*Helianthus annuus*) and Tobacco (*Nicotiana tabacum*) revealed an increase in tissue mass after 6 weeks in the presence of moon soil. The tobacco tissue cultures also revealed an increase in the degree of pigmentation after their exposure to the lunar soil. At present, the histological and biochemical compositions of those cultures which responded to the lunar soil of Apollo 11 and 12 are being investigated. These results will be reported. Comparisons will be made of the morphological and the structural characteristics of the tissue cultures of Apollo 11 and 12.

9:40 **Sweet, H. C., C. H. Walkinshaw,** and **S. Venketeswaran.** Brown and Root-Northrop, USDA (Forest Service), NASA Lunar Receiving Laboratory, and University of Houston, Houston, Texas.—REACTIONS OF SEVERAL SPECIES OF LOWER PLANTS TO LUNAR MATERIAL.—Samples of lunar material collected during the Apollo missions were examined at the Lunar Receiving Laboratory for the presence of plant and animal pathogens. No deleterious effects were noted in the 31 species of plants which had been challenged with a pooled sample of crushed lunar rock and lunar fines. However, several species of lower plants including ferns and liverworts exhibited a striking increase in green pigmentation and tissue mass when in contact with the lunar material. These findings will be discussed in relation to the inorganic chemical analysis of the Apollo 11 and 12 lunar samples.

10:00 **Mahlberg, P., K. Olson,** and **C. Walkinshaw.** Indiana University, Bloomington; Hiram Scott College, Scottsbluff, Nebraska; Lunar Receiving Laboratory, Texas.—DEVELOPMENT OF PERIPHERAL VACUOLES IN PLANT CELLS.—The vacuolar apparatus of plant cells consists of two distinct features: the large central vacuole and peripheral vacuoles which are derived from invaginations of the plasma membrane. Peripheral vacuoles are conspicuous structures in both living and fixed hair or filament cells of *Tradescantia virginiana* L. They occur as more or less spherical structures along the peripheral cytoplasm and can be recognized as projections into the central vacuole. These structures are variable in size and number within a cell and can represent a significant volume of the vacuole. Peripheral vacuoles most frequently are observed in motion with the streaming cytoplasm although their velocity is usually somewhat slower than that of the cytoplasmic organelles. Ultrastructural studies

TWO POPULATIONS OF ALIPHATIC HYDROCARBONS OF TERATOMA AND HABITUATED TISSUE CULTURES OF TOBACCO

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ABSTRACT

Teratoma and habituated tissue cultures of tobacco grown under identical conditions were examined for the presence of paraffinic hydrocarbons. The teratoma tissues contained n-C_{29}, 2-methyl C_{30} (iso C_{31}) and n-C_{31} as the major alkane components and their distribution pattern was qualitatively identical to the seedling tissue alkanes (C_{22} - C_{34}). Habituated tissues contained a different population of alkanes ranging in carbon chain length from C_{17} to C_{28}. The predominant alkane components were n-C_{23}, n-C_{22}, and n-C_{24} in decreasing concentrations respectively.

A tissue culture system is presented where the Population I hydrocarbons (C_{16} - C_{28}) are present and synthesized separately and independently of Population II hydrocarbons (C_{27} - C_{34}).

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INTRODUCTION

Paraffinic hydrocarbons are found in tissues of plants from all divisions of the plant kingdom. The distribution and metabolism of these compounds in plant tissues have been extensively reviewed (1-5), but the exact physiological importances of these hydrophobic components remains uncertain (5). Emphasis has been placed on the hydrocarbon components as part of waxy layers on the external surfaces of leaves, stems, and floral parts exposed to the atmosphere. Only recently has attention been given to the internal population of relatively short chain alkanes (6).

The surface and cellular alkanes of differentiated plant systems have received greatest attention whereas those of non-differentiated tissue cultures have not been examined. The teratoma and habituated tissue cultures of tobacco represent excellent systems to illustrate the metabolic differences between differentiated and non-differentiated tissues. The teratoma tissue is of tumerous origin (crown gall disease caused by Agrobacterium tumefaciens) and maintains its pathological nature under normal conditions of culture while having a large degree of differentiation (shoots and leaves). The habituated tissue is of normal origin, only proliferates, and does not show any organ differentiation (7,8).

This paper reports the presence and distributions of two populations of paraffinic hydrocarbons as they occur separately in the teratoma and habituated tissue cultures of tobacco. The developmental aspects of tissue cultures of tobacco with respect to hydrocarbon synthesis are discussed.

RESULTS AND DISCUSSION

The paraffinic hydrocarbons of 4-6 week old tobacco seedling tissues are both qualitatively and quantitatively similar to that reported previously by Kaneda (9,10). There are three homologous series consisting of the normal, 2-methyl, and 3-methyl substituted branched-chain alkanes. A unique pattern of branched-chain isomers exists. Methyl substitution at the number two position is found only in the odd-numbered carbon compounds while methyl substitution at the number three position occurs only in the even-numbered carbon compounds.

Total hydrocarbon concentrations of the tobacco seedling and teratoma tissues are 1557.0 and 156.8 μg/g dry weight respectively. The ratio of normal to branched-chain alkanes in the seedling and teratoma tissues is approximately 1.1:1 and 2:1 respectively, revealing that teratoma tissues contain higher relative concentrations of straight chain isomers than the seedling tissue.

The three predominant hydrocarbon components of seedling tissues used for this study are n-C_{31}, n-C_{33}, and 3-methyl C_{31} (2-methyl C_{30} is found in concentrations slightly less than 3-methyl C_{31}) in decreasing concentrations respectively (TABLE I). This corresponds closely to the results obtained by Kaneda (9,10) who found that n-C_{31}, 2-methyl C_{30}, and n-C_{33} in decreasing concentrations respectively were the predominant alkanes. The slight variation in relative proportions is probably due to differences in age of the tissues and methods of extraction.

The hydrocarbon fraction of teratoma tissue culture samples contained a distribution of alkanes identical to the seedling tissues. The predominant components of this tissue were n-C_{29}, 2-methyl C_{30}, and C_{31} in decreasing concentrations respectively (TABLE I). This

is similar to the seedling with the exception that c-C_{29} appears in the highest concentrations while n-C_{33} is not a major component of the teratoma tissues. With the exception of 3-methyl C_{31}, odd-numbered carbon compounds are predominant in both the seedling and teratoma tissues. The predominance of odd-numbered hydrocarbon chain lengths is typical of most higher plant species (2-5).

When the hydrocarbon components from total lipid extracts of habituated tissue culture samples were examined, a distribution was found which is very similar to that reported for the internal alkanes of spinach leaf tissues. Very low concentrations of alkanes (44.5 µg/g dry weight of tissue) were present (TABLE I). Alkanes were present in concentrations less than 1/3 that of the teratoma tissue alkanes and 1/35th. that of the seedling tissue alkanes. Carbon chain lengths range from C_{17} to C_{28} with no predominance of the odd-numbered carbon chain lengths (TABLE I). Major alkane components of habituated tissue culture samples are C_{23}, C_{22}, and C_{24} in decreasing concentrations respectively (TABLE I). Several compounds were resolved by GLC in such low concentrations that structural confirmations could not be made. These components were located between the major alkanes of the chromatogram and probably represent specifically branched or unsaturated isomers.

Comparison of the hydrocarbon fractions of the partially differentiated teratoma tissues and seedling tissues reveal no qualitative differences while teratoma tissues contain significantly less alkanes than the seedling tissues. On the other hand, alkanes of the non-differentiated habituated tissues differ both qualitatively and quantitatively from the teratoma and seedling tissues.

A distribution of hydrocarbons almost identical to the internal hydrocarbons of spinach leaves reported by Kaneda (6) was found. Internal hydrocarbon distributions are probably of universal occurrence but have been overlooked because of the methods of extraction and that high concentrations of higher molecular weight alkanes mask their presence during analysis. For example, Weete et al. (11) found a very similar distribution of alkanes in the root tissues of the halophyte Salicornia bigelovii which has a cortical surface with no cuticular waxy coating. Thus it appears that many higher plants contain two populations of hydrocarbons: Population I, which are found in low concentrations with carbon chain lengths of C_{16} to C_{28} with no odd-numbered carbon chain predominance and located in the external waxy coating and consisting predominantly of higher molecular weight alkanes with their major components containing odd-numbered carbon chain lengths.

A comparison of the hydrocarbon distributions of the teratoma (differentiated) and habituated (non-differentiated) tissues illustrates this two population concept and further suggests the presence of two enzyme systems for their synthesis and/or two loci of synthesis. A composite of the teratoma and habituated tissue hydrocarbon components reveals a bimodal distribution: Population I of the habituated and Population II of the teratoma tissues. The non-differentiated habituated tissues apparently does not contain the enzyme system or metabolic machinery for activation of an enzyme system necessary for synthesis of the Population II hydrocarbons.

In summary, the data presented in this paper demonstrates the presence of two individual populations of paraffinic hydrocarbons appearing separately in teratoma and habituated tissue cultures of tobacco. Of greater significance, however, is that a tissue culture

system is presented that is capable of synthesizing only the Population I hydrocarbons providing an ideal system for answering questions concerning the developmental aspects of activation and localization of the biogenisis of paraffinic hydrocarbons.

METHODS AND MATERIALS

Tissue cultures and seedlings: Teratoma and habituated tissue cultures were grown by the methods previously described by Chen and Venketeswaran (8,12,13). Seedling tissues (4-6 weeks old) were grown in soil with 12 hour alternating illumination. All tissues were harvested, dried by lypholyzation, weighed, and stored at -4°C prior to lipid extraction.

Extraction and sample preparation procedures: One gram dry weight of the lypholyzed teratoma and habituated tissues and 0.42 g (dry weight) of the seedling tissues were extracted for total lipid extract was subjected to alkaline hydrolysis described by Wilde and Stewart (15). The hydrolysate was first washed with 3 volumes (20 ml each) of n-haptane to remove the non-saponifiable material. The lower aqueous phase was adjusted to pH 2 and washed with 3 volumes (20 ml each) of diethyl ether. Both organic phases were taken to dryness under nitrogen with gentle heating. The non-saponifiable fraction was taken up in n-heptane and placed on top of a pretreated (150°C of 1 hour) silica gel column (1 x 20 cm). The hydrocarbon components were eluted with 3 volumes (20 ml each) of n-heptane. The hydrocarbon fraction was taken to dryness prior to analysis by gas chromatography and GLC-mass spectrometry combination.

GLC and GLC-mass spectrometry: The hydrocarbon fraction was taken up in 20-50 µl of n-heptane and 1/20 to 1/50 of the total

fraction was injected directly into a Perkin Elmer 900 gas chromatograph equipped with a 50 ft. x 0.03 in stainless steel capillary column coated with SE-30 (Applied Science Laboratories, State College, Pa.). The oven temperature was programmed from 100° - 250°C at 10°C per minute. Solvent blanks were run to ensure the absence of contaminants.

Structural confirmations of each paraffinic hydrocarbon was made by mass spectrometry. The mass spectra of the alkanes were identical to those previously published.

Acknowledgements: The authors wish to express their appreciation to Miss Jo Ann Fowler for her technical assistance and Dr. Peter K. Chen for supplying teratoma tissue cultures. This research was supported by grant-in-aid from the Brown-Hazen Fund, Research Corporation, California and N.A.S.A. Grant No. NAS-9-8264. This manuscript was prepared at the Lunar Science Institute, Houston, Texas, under the joint support of the Universities Space Research Association, Charlottesville, Virginia, and the National Aeronautics and Space Administration Manned Spacecraft Center, Houston, Texas, under contract No. NSR 09-051-001.

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TABLE I. Hydrocarbon Components of Habituated and Teratoma Tissue Cultures and Seedling Tissues of Tobacco

	HABITUATED		TERATOMA		SEEDLING	
HYDROCARBON	%	µg/g*	%	µg/g*	%	µg/g*
C_{17}	4.8	2.0	--	--	--	--
C_{18}	5.7	2.5	--	--	--	--
C_{19}	4.6	2.0	--	--	--	--
C_{20}	7.6	3.5	--	--	--	--
C_{21}	6.9	3.0	--	--	1.0	16.1
C_{22}	12.3	6.0	1.6	2.4	0.8	10.7
C_{23}	19.1	9.5	3.5	5.5	1.0	16.1
C_{24}	11.8	5.5	3.2	5.0	0.9	14.3
C_{25}	8.1	3.5	3.3	5.3	1.2	19.8
C_{26}	5.4	2.5	3.2	5.0	0.9	14.3
2-me-C_{26}	--	--	1.5	2.4	trace	trace
C_{27}	5.0	2.0	5.9	9.5	4.8	75.0
3-me-C_{27}	--	--	2.0	3.2	1.8	28.6
C_{28}	5.2	2.5	6.0	9.5	1.9	30.3
2-me-C_{28}	--	--	9.5	15.2	7.5	118.0
C_{29}	--	--	12.3	18.2	8.6	135.5
3-me-C_{29}	--	--	7.2	11.4	9.4	147.5
C_{30}	--	--	4.3	6.6	3.0	47.4
2-me-C_{30}	--	--	10.9	17.2	10.1	158.7
C_{31}	--	--	10.3	16.4	14.7	228.2
3-me-C_{31}	--	--	5.0	7.9	10.4	164.0
C_{32}	--	--	2.8	4.5	3.1	47.7
2-me-C_{32}	--	--	3.2	5.0	5.1	79.8
C_{33}	--	--	4.2	6.6	12.5	195.0
3-me-C_{33}	--	--	trace	trace	trace	trace
C_{34}	--	--	--	--	trace	trace
TOTAL HYDROCARBON		44.5		156.8		1557.0

*Micrograms of alkane per gram dry weight of tissue.

APPENDIX 1

PROGRESS REPORTS

The Contractor provided monthly reports starting September 30, 1968 until November 30, 1968, and was informed that instead of monthly reports, a final report should be presented at the end of the contract period. Copies of the 3 earlier reports are included in this Appendix 1 as part of this final report.

First Monthly Progress Report

Contract No. NAS 9-8264

Contractor: University of Houston
3801 Cullen Blvd
Houston, Texas 77004

DEVELOPMENT OF LUNAR RECEIVING LABORATORY BIOLOGICAL PROTOCOL

September 30, 1968

S. Venketeswaran
Associate Professor
in Biology

This contract was started as a new one on July 1, 1968 consisting of additional tasks relative to an early contract NAS 9-6822 entitled "Development of Lunar Receiving Laboratory Biological Protocol."

The present report is the first of a series of monthly reports on the new Contract No. NAS. 9-8264 entitled "Experiments and Operational Procedures for Developing Germ-Free Seeds, Seedlings, Plants, Tissues and Cell Lines for the Lunar Receiving Laboratory." Data required to establish norms of certain algae and higher plant tissue culture growth characteristics are in progress.

PART 1

The following algae are in culture on agar base slants in the amounts shown:

Chlorella miniata #400	40 slants
Chlorococcum aplanosporum	35 slants
Trebouxia	109 slants
Chlorella vulgaris #580	34 slants
Chlorella vulgaris #262	24 slants
Anabaena	5 slants
Chlamydamonas #9	8 slants
Oscillatoria	6 slants

In liquid culture:

Nostoc	37 tubes
Anabaena	25 tubes
Oscillatoria	10 tubes
Anacystis nidulans	8 tubes

All of these organisms were transferred to new media under sterile conditions July 28, 1968.

The media used for maintaining the algae on solid slants is the one described by Murashige and Skoog (1962).

The algae are maintained in liquid suspension in Kratz and Meyer's modification (1955) of Chu's;medium, to which is added one ml of Hunter's (1955) A-5 micro-elements.

PART II.

The following section describes in detail the progress made in establishing some of the basic growth characteristics of the following tissues: rice, soybean, tobacco, and Haploppapus. These four tissues are the first of several tissue types to be studied in this series. The characteristics to be studied are DNA content, RNA content, and total protein content of tissues of known age.

Tobacco tissue was used to run the preliminary tests described here. The purpose of the data to be described is twofold. Firstly, to obtain some concept of the range of sensitivity of the DNA and total nucleic acid analyses, and secondly, to determine the quantity and dilutions which must be made on a given sample in order to obtain the most reliable answers from each sample.

The procedure which is described is a combination of the Schneider (1951) method and the Schmidt and Thannhauser method (1945).

The total nucleic acid content was measured using a model DB Beckman spectrophotometer at 260 mμ. The DNA content was estimated by the color reaction with diphenylamine. Absorbance readings for DNA estimation only were taken at 600 mμ. All measurements were standardized against a commercially available fish sperm DNA which was considered to be 100% pure.

The procedure used in the separation and analysis of both the total nucleic acids and the DNA as performed on lyphilized tobacco tissue is as follows:

Removal of lipid and acid soluble substances:

1. Approximately 100 mg of lyphilized tobacco tissue is mixed with 15 ml of chilled 10% perchloric acid (PCA). After agitation, centifruge at 3000 x g, 0°C for 15 minutes. Discard supernatant.

2. Wash pellet with 10 ml 95% ethanol and centrifuge at 3000 x g for 10 minutes at 0°C. Discard supernatant. Repeat the washing two times more.
3. Wash pellet with 10 ml 50/50 ethanol/ethyl ether. Centrifuge at 8000 x g at 0°C for 10 minutes. Discard supernatant.
4. Wash pellet with 10 ml 100% ether. Centrifuge, discarding the supernatant. Centrifuge at 8000 x g at 0°C for 15 minutes.
. Allow pellet to air dry.

Note: All of the above procedures are performed in an ice bath at 4°C.

DNA and total nucleic acid estimation:

1. The dry pellet is suspended in 5 ml of 5% perchloric acid. It is then boiled in a water bath at 70°C (PCA boiling point) for 15 minutes. Use glass or metal centrifuge tubes if possible for this and the succeeding steps.
2. Centrifuge the boiled suspension at 8000 x g for 10 minutes. Save the supernatant.
3. Repeat above two steps two more times pooling the supernatants.
4. Dilute 1.5 ml of the pooled supernatant with 1.5 ml 5% PCA.
5. Place 3 ml of this diluted supernatant in a quartz cuvette and standardize against DNA standard at 260 mμ.
6. To 1 ml of the pooled supernatant, add 2 ml of diphenylamine reagent. Heat for 10 minutes in a water bath. Read absorbance at 600 mμ and standardize against the DNA standard.

The data which were collected from this first run are shown in Table 1. Statistical analyses for significance have not been run on these data as yet because the computer program to handle the computation is not yet completed. It appears, however, that by following this method carefully, easily reproducible answers can be obtained.

Color reaction of diphenylamine does not develop with time so the timing of the reaction is not needed.

During the first run of the DNA standard curves, it became apparent that the sensitivities of the two measuring techniques differed considerably. This is the reason for a concentration step in the procedure leading to the diphenylamine reaction. The range and dilutions for standards and samples are listed below.

1. U.V. method for total nucleic acids:
   a. Standard: 25 µg/ml to 75 µg/ml
   b. Sample: dilute sample volume 1:1
2. Diphenylamine color reaction:
   a. Standard: 50 µg/ml to 200 µg/ml
   b. Sample: concentrate sample to ¼ original volume

Recipe

Diphenylamine reagent:

1 gm diphenylamine
100 ml glacial acetic acid
2.75 concentrated sulfuric acid

Note: Reagent must be made up fresh each time.

Table 1. Total nucleic acid content and DNA content of lyophalized tobacco tissues in culture after 21 days' growth.

	Dry Weight	Total Nucleic Acid conc. (260 mμ)	Total DNA conc. (600 mμ)	Total Weight of Nucleic Acids in Sample	Total Weight DNA in Sample	mg Nucleic Acid/G.D.W.	mgDNA G.D.W
Sample A	0.1116 g	24 μg/ml		480 μg		4.3011 mg/g	
Sample B	0.1391 g	28.5 μg/ml		570 μg		4.0977 mg/g	
Sample A	0.1116 g		12 μg/ml		240 μg		2.15 mg/g
Sample B	0.1391 g		20 μg/ml		400 μg		2.88 mg/g

Original volume of all samples = 20 ml.

TNA is about 1/3 that obtained by Guinn (1966) in cotton leaves.

DNA mg/g D.W. is comparable with that obtained by Guinn (1966) in cotton leaves.

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Second Monthly Progress Report

Contract No. NAS 9-8264

Contractor University of Houston
3801 Cullen Blvd
Houston, Texas 77004

DEVELOPMENT OF LUNAR RECEIVING LABORATORY BIOLOGICAL PROTOCOL

S. Venketeswaran
Associate Professor

October 1968

This is the 2nd. monthly report on the progress of the contract No. 9-8264 entitled "Development of Lunar Receiving Biological Protocol" in our laboratory for the month of October.

Algae

The following algae are presently being maintained in aseptic cultures for the purpose of determining certain growth characteristics (protein content, DNA content, and RNA content):

Chlorella miniata #490	39 slants
Anacystis nidulans	60 slants
Phaeodactylum tricornutum	6 slants
Porphyridium cruentum	34 slants

Chlorella miniata #490 are being maintained on solid slants of Murashige and Skoog's media (1962). Anacystis nidulans are being maintained on Kratz and Meyer's (1955) modification of Chu's medium which has been solidified by the addition of 1.5% Bacto-agar. Phaeodactylum tricornutum are being cultured on a modification of Provasoli's medium ASP-2 (Mann, 1968).

Porphyridium cruentum are being maintained on a modification of the media proposed by Richard Starr (1964). The composition of the medium is as follows:

Porphyridium Agar. For each 500 ml of medium required

Pyrex-distilled water	200.0 ml
Naturel sea water	250.0 ml
Soil-water supernatant	50.0 ml
Yeast extract	0.5 g
Tryptone	0.5 g
Agar	7.5 g

Other algae are also maintained in culture on Murashige and Skoog's medium:

Chlorella vulgaris #580	75 slants
Chlorella vulgaris #262	18 slants
Chlorococcum aplanosporum	40 slants
Trebouxia	80 slants
Anabaena	20 slants
Chlamydamonas #9	16 slants
Chlamydamonas #10	20 slants

RNA Analysis of Tissue Cultures

RNA determination was begun this month and some preliminary results were obtained using soybean tissue.

Procedure:

1. Delipidized pellet is placed in 10 ml of 1N KOH and incubated at 37°C for 20 hours
2. Add 1 ml (con) HCl
3. Add 5 ml 5% TCA
4. Centrifuge at 1,200 Rpm for 15 minutes
5. Add 5 ml 5% TCA and recentrifuge
6. Decant supernatant into a new tube
7. Use .2 ml supernatant
   1.3 ml H_{20}
   1.5 ml Orcinol reagent*
8. Boil 20 minutes in a H_{20} bath
9. Read at 600 mμ

*Orcinol reagent is made by putting 1 gm Orcinol and .5g F_eCl_3 into solution in 100 ml of con HCl.

Results:

Wt. of Sample	OD	µg as read from Standard Curve	mg RNA/gm dry wt. Soybean Tissue
.1537	.381	47.0	65.5
.1397	.399	49.0	73.8
.1730	.430	52.0	63.1
.1618	.415	50.0	64.8
.1618	.470	60.5	78.3
.1675	.385	45.0	56.4
.1675	.355	42.5	54.5
.1680	.510	61.0	78.3
.1680	.430	51.0	60.4
.1357	.310	38.0	58.8
.1357	.270	32.0	49.6

Average 65.8 mg RNA/gm dry wt. of Soybean Tissue

or

$$\frac{.0658}{1.0000} \times 100 = 6.5\% \text{ RNA}$$

Conclusions: There is a large deviation in the amount of RNA read for different samples. Preliminary tests to develop proficiency in this technique before weekly analysis are begun and detailed analysis will be reported later.

Third Monthly Progress Report

Contract No. NAS 9-8264

Contractor University of Houston
3801 Cullen Blvd
Houston, Texas 77004

DEVELOPMENT OF LUNAR RECEIVING LABORATORY BIOLOGICAL PROTOCOL

S. Venketeswaran
Associat Professor
of Biology

November 30, 1968

The present report is the third of a series of monthly reports on the new contract No NAS 9-8264 entitled "Development of Lunar Receiving Laboratory Biological Protocol." Data required to establish norms of certain algae and higher plant tissue culture are in progress.

SEED GERMINATION

Seed germination was carried out for Brassica oleracea var. Greenback, Lycopersicon esculentum var. Homestead, and Hordeum vulgare var. Moore. The criteria of germination was when the seed coats break open and the young embryo comes out of the cotyledon. The percent germination was recorded daily for six petri dishes each containing 25 seeds and can be seen below.

Percent Germination Cabbage

Day	Dish#1	Dish#2	Dish#3	Dish#4	Dish#5
1	0	0	0	0	0
2	52	52	48	60	32
3	76	60	80	76	52
4	100	92	100	96	80
5		92		100	88
6		100			92
7					92
8					96

Percent Germination Tomato

Day	Dish#1	Dish#2	Dish#3	Dish#4	Dish#5
1	0	0	0	0	0
2	4	0	0	0	0
3	80	76	60	64	64
4	92	92	92	76	80
5	92	92	96	96	88
6	92	92	100	96	96
7	92	92		100	96
8	92				96

Percent Germination Oat*

Day	Dish#1	Dish#2	Dish#3	Dish#4	Dish#5
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	4
4	0	0	0	0	4
5	8	4	0	0	8
6	8	4	0	0	16
7	8	8	0	8	16
8	8	8	0	8	16

*Poor germination could have resulted from inhibitors on the husk of the oat seeds. Future attempts are made by dehusking the seeds.

TISSUE CULTURE

Growth experiments were carried out for the tissues of Soybean, Habituated Tobacco and Haplopappus. Fresh weights were obtained as can be seen below.

Soybean (10 replicates	0 Days	10 Days	20 Days	30 Days
	gm	gm	gm	gm
Mean Fresh Weight	3.5053	6.9954	7.8668	8.5800
SD	±0.6165	±1.9904	±1.2294	±1.6750
SE	±0.2191	±0.6294	±0.3888	±0.5297

Habituated Tobacco (10 replicates)				
Mean Fresh Weight	4.9021	7.6704	12.736	15.7800
SD	±0.7484	±1.9855	±2.5775	±1.8400
SE	±0.2367	±0.6279	0.8150	±0.3744

Haplopappus (10 replicates)			
Mean Fresh Weight	3.1641	8.3987	10.0341
SD	±1.3716	±1.6103	±1.4527
SE	±0.433	±0.5092	±0.4594

Dry weights and analyses of the data will be submitted at a later date.

NUCLEIC ACID ANALYSIS

The DNA content was determined for tissues of Rice, Tobacco and Soybean by extraction with hot TCA and reaction with Diphenylamine reagent. The details of this procedure were reported earlier (second monthly report). The following results were obtained.

RICE - DNA IN MG/GRAM (DRY WT.) OF TISSUE

Sample wt. (dry)	OD	DNA µg as read from standard curve	mg DNA/gram dry wt.
.1000 g	.17	140 µg	10.52
.1000 g	.155	127 µg	9.54
.1000 g	.167	135 µg	10.15
.1000 g	.178	145 µg	10.90
.1000 g	.160	130 µg	9.84
.1000 g	.125	105 µg	7.89
.0820 g	.18	150 µg	13.13
.0820 g	.168	140 µg	12.72
		Average	10.65mg DNA g dry wt

TOBACCO - DNA IN MG/GRAM (DRY WT.) OF TISSUE

Sample wt.	OD	µg as read from standard curve	mg DNA/gram dry wt
.1093	.2	51.5	3.70
.1207	.112	26.5	1.76
.1097	.145	34	2.31
.1279	.23	61	3.80
		Average	2.89mg DNA g dry wt

SOYBEAN - DNA IN MG/GM (DRY WT.) OF TISSUE

Sample wt.	OD	µg as read from standard curve	mg DNA/g Soybean
.1025	.240	135 µg	12.70
.1025	.260	150 µg	14.70
.1025	.245	140 µg	13.60
.1025	.215	115 µg	11.20
.1019	.26	150 µg	14.72
.1019	.25	135 µg	13.20
.1019	.27	160 µg	15.73
		Average	13.69mg/gm dry wt.

PROTEIN DETERMINATION

Protein analyses were carried out on Lypholized tissues of Soybean, Tobacco and Rice using Lowry's method (1951) of protein determination.

Reagents

A = 4% Na_2CO_3 in 0.1 N Na OH

B = 4% Na Tartrate

C = 2% Cµ SO_4

D = Folin-Phenol reagent diluted 1:1

E.= 100 ml of A + 1 ml B + 1m C freshly mixed each time.

Procedure: Lypholized samples are ground in H_2O, and centrifuged at 12,000 RPM for 15 minutes. Readjust the normality to .1 N by the addition of 1 N Na OH. The supernatant is used for analysis.

5 ml of E is added to the sample and allowed to stand at room temperature for 15 minutes.

.5 ml of D is added, mixed immediately and allowed to stand for 30 minutes. This was read on a model DB Beckman Spectrophotometer at 750 mµ.

Results:

Trial I.

	Standard (BSA)	OD
1	300 µg/ml	0.81
2	225 µg/ml	0.63
3	150 µg/ml	0.47
4	75 µg/ml	0.281

Trial II.

	Standard (BSA)	OD
1	210 µg/ml	0.54
2	160 µg/ml	0.415
3	105 µg/ml	0.265
4	52.5 µg/ml	0.135

Soybean

Trial I.

Sample wt.	OD	µg Protein	µg Protein/mg Tissue	% Protein
3000 µg	0.68	244 µg	81.3	8.1
1500 µg	0.38	117.5 µg	78.3	7.9
750 µg	0.25	60 µg	80.0	8.0

Trial II.

3000 µg	0.60	236 µg	78.6	7.8
1500 µg	0.30	117.5 µg	78.3	7.8
750 µg	0.15	57.5 µg	76.6	7.6
			Average 78.8	Average 7.9

Tobacco

Trial I.

Sample Wt.	OD	µg Protein	µg Protein/mg Tissue	%Protein
3000 µg	0.31	82.5	27.5	2.7
1500 µg	0.19	35	23.3	2.3
750 µg	0.09			

Trial II.

3000 µg	0.21	80.0	26.6	2.6
1500 µg	0.08	31.0	20.6	2.0
750 µg	0.03	15.5	20.7	2.1
		Average	23.7	Average 2.3

Rice

Trial I.

Sample Wt.	OD	µg Protein	µg Protein/mg Tissue	% Protein
3000 µg	0.83	310	103.3	10.3
1500 µg	0.50	167	111.3	11.1
750 µg	0.283	77	102.9	10.3

Trial II.

3000 µg	0.80	300	100.0	10.0
1500 µg	0.46	160	106.6	10.7
750 µg	0.205	85	113.3	11.3
		Average	106.2	Average 10.6

LITERATURE

1. Lowry, O. H., Rosenbrough, N. R., Farr, A. L. and Randall, R. J. (1951). Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193:265-275.

2. Venketeswaran, S. (1968). Development of Lunar Receiving Laboratory Biological Protocol. 2nd. Monthly Progress Report on NAS 9-8264 Contract.

Appendix 2

MEDIA COMPOSITION

During the contract period various culture media were tested in order to get the best growth of the tissue cultures. The compositions of these media were reported in communications during the contract period. In the following pages, the composition of the culture media as employed at the end of this present contract, viz., September 30, 1968, are given for each of the plant species.

MACHLIS MEDIUM (AS MODIFIED BY MILLER AND MACHLIS 1967)

TISSUES CULTURED ON MEDIUM: SPHAEROCARPAS DONNELLI

pH 5.5-6.0

		Stock Solution, gm/liter	Stock Solution ml/liter of medium	Final Concentration mg/liter
(1)	Major Solution			
	KH_2PO_4	136.0	10.0	1360.0
	$MgSO_4.7H_2O$	25.0	10.0	250.0
	$CaCl_2$	11.0	10.0	110.0
	KNO_3	152.0	10.0	1520.0
(2)	Minor Solution			
	H_3BO_3	10.0		100.0
	$CuSO_4.5H_2O$	1.5	1.0	15
	EDTA	50		500
	$ZnSO_4.7H_2O$	22		220
	$CaCl_2$	5.0		50
	$MnCl_2.4H_2O$	5.0		50
	$FeSO_4.7H_2O$	5.0		50
	$CoCl_2.6H_2O$	1.5		15
	$(NH_4)_6Mo_7O_{24}.4H_2O$	1.0		10
(3)	Sucrose	15		1.5%
(4)	Agar	8-10		8-10%

To make the minor stock solution. Add all chemicals in order to 75 ml of H_2O, bring this to the boil and then cool slightly. Adjust the pH to approximately 6.5 with KOH pellets and dilute the resulting solution to 100 ml.

HOAGLAND'S MEDIUM (¼ STRENGTH)

TISSUES CULTURED ON MEDIUM: MARCHANTIA

pH 6.5-7.5

	Major Solution	Stock Solution, gm/liter	Stock Solution ml/liter of medium	Final Concentration mg/liter
(1)	$Ca(NO_3)_2$	164	1.25	205
(2)	KNO_3	101	1.25	126.25
(3)	KH_2PO_4	136	0.5	68
(4)	$MgSO_4$	120	0.25	40
(5)	$FeCl_3.6H_2O$	1.0	1.0	1.0
	Minor Solution			
(6)	H_3BO_3	2.86		2.8
	$MnCl_2.4H_2O$	1.81	1.0	1.81
	$ZnSO_4.7H_2O$	0.22		0.22
	$CuSO_4.5H_2O$	0.08		0.08
	$H_2MoO_4.H_2O$	0.02		0.02
(7)	Sucrose	20		2%
(8)	Agar	10		1%
	H_2O			to make 1 liter

S.V. MEDIUM AND IAA AND KINETIN

TISSUE CULTURED ON MEDIUM: DANCUS CAROTA

pH 5.5-6.0

	Major Solution	Stock Solution, gm/liter	Stock Solution ml/liter of medium	Final Concentration mg/liter
(1)	NH_4NO_3	82.5	20.0	1650.0
	KNO_3	95.0		1900.0
(2)	$CaCl_2.2H_2O$	88.0	5.0	440.0
(3)	$MgSO_4.7H_2O$	74.0	5.0	370.0
	KH_2PO_4	34.0		170.0
(4)	Na_2EDTA	7.45	5.0	37.3
	$FeSO_4.7H_2O$	5.57		27.8
	Micro Nutrients			
(5)	$MnSO_4.H_2O$	4.5		4.5
	$ZnSO_4.7H_2O$	1.5		1.5
	H_3BO_3	1.5		1.5
	$CuSO_4.5H_2O$	0.04	1.0	0.04
	$Na_2MoO_4.2H_2O$	0.25		0.25
	$CoCl_3.6H_2O$	0.005		0.005
	$AlCl_3$	0.003		0.003
	$NiCl_2.6H_2O$	0.003		0.003
	KI	0.001		0.001

S.V. MEDIUM AND IAA AND KINETIN

TISSUE CULTURED ON MEDIUM DANCUS CAROTA (continued)

pH 5.5-6.0

	Major Solution	Stock Solution, gm/liter	Stock Solution ml/liter of medium	Final Concentration mg/liter
(6)	Vitamins and Amino Acids			
	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1	1.0	0.1
	Pyridoxine HCl	0.1		0.1
	Glycine	3.0		3.0
(7)	NAA	0.175	1.0	0.175
(8)	2,4-D	0.221	1.0	0.221
(9)	Yeast Extract	0.1		0.1%
(10)	Coconut Milk		100.0	10%
(11)	Ascorbic Acid	50.0	1.0	50.0
(12)	Kinetin	0.04	1.0	0.04
(13)	IAA	2.0	1.0	2.0

HELLER'S MEDIUM

TISSUES CULTURED ON MEDIUM: RICE

pH: 5.5-6.0

(1) Major Solution	Stock Solution, gm/liter	Stock Solution ml/liter of medium	Final concentration, mg/liter
KCl	100.0	7.5	750.0
$NaNO_3$	100.00	6.0	600.0
$MgSO_4.7H_2O$	100.0	2.5	250.0
$NaH_2PO_4.H_2O$	10.0	12.5	125.0
$CaCl_2.2H_2O$	10.0	7.5	75.0
$FeCl_3.6H_2O$	1.0	1.0	1.0
(2) Minor Solution			
$ZnSO_4.7H_2O$	1.0		1.0
H_3BO_3	1.0	1.0	1.0
$MnSO_4.4H_2O$	0.01		0.01
$CuSO_4.4H_2O$	0.03		0.03
$AlCl_3$	0.03		0.03
$NiCl_2.6H_2O$	0.03		0.03
KI	0.01		0.01

HELLER'S MEDIUM

TISSUES CULTURED ON MEDIUM: RICE (continued)

pH: 5.5-6.0

	(3) Vitamins and Amino acids	Stock Solution, gm/liter	Stock Solution ml/liter of medium	Final concentration, mg/liter
	Glycine	3.0		3.0
	Thiamine HCl	0.5	1.0	0.5
	Nicotinic acid	0.5		0.5
	Pyridoxine	0.5		0.5
(4)	D,L-Tryptophane	60.0	1.0	60.0
(5)	2,4-D	2.0	1.0	2.0
(6)	Coconut milk		100.00	10%
(7)	Yeast extract			0.5%
(8)	Sucrose		30.0 g/l	3%
(9)	Agar		8.0-10.0 g/l	0.8%-1%

"The macroelements (KCl, $NaNO_3$, $Na_2SO_4.7H_2O$) are very soluble (100 g/l but these stock solutions are relatively concentrated. The others ($NaH_2PO_4.H_2O$, and $CaCl_2.2H_2O$) are less soluble and so should be kept in more dilute solutions (10 g/l).

The minor elements with exception of $FeCl_3.6H_2O$ are grouped together into one solution and one ml of the stock solution is used to make a liter of media. The feric chloride is kept in a separate solution because it causes precipitation when grouped with other minor elements." (R. J. Gautheret, 1959).

MURASHIGE AND SKOOG MEDIUM

TISSUE CULTURED ON MEDIUM: EUPHORBIA

	Major Solution	Stock Solution, gm/liter	Stock Solution ml/liter of medium	Final concentration mg/liter
(1)	NH_4NO_3	16.5		1650.0
	KNO_3	19.0	100.0	1900.0
	$CaCl_2.2H_2O$	4.4		440.0
	$MgSO_4.7H_2O$	3.7		370.0
	KH_2PO_4	1.7		170.0
(2)	Na_2EDTA	7.45	5.0	37.3
	$FeSO_4.7H_2O$	5.57		27.8
	Minor Solution			
(3)	HBO_3	6.2		6.2
	$MnSO_4.4H_2O$	22.3		22.3
	$ZnSO_4.4H_2O$	8.6	1.0	8.6
	KI	0.83		0.83
	$Na_2MoO_4.2H_2O$	0.25		0.25
	$CuSO_4.5H_2O$	0.025		0.025
	$CoCl_2.6H_2O$	0.025		0.025
	Vitamins and Amino acids			
(4)	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1	1.0	0.1
	Pyridoxine HCl	0.1		0.1
	Kinetin	0.5		0.5
(5)	IAA	2.0	1.0	2.0
(6)	Kinetin	.04	1.0	0.04
(7)	Ascorbic acid	50.0	1.0	50.0
(8)	Myoinositol	100.0	1.0	100.0
(9)	Sucrose	30.0		3%
(10)	Agar	10.0		1%

WHITE'S IX MEDIUM

TISSUES CULTURED ON MEDIUM: CORN

pH 6.1-7.0

	Macroelements	Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration, mg/liter
(1)	$Ca(NO_3)_2.4H_2O$	6.0		300.0
	Na_2SO_4	4.0	50.0	200.0
	KNO_3	1.6		80.0
	KCl	1.3		65.0
	$NaH_2PO_4.H_2O$	0.33		16.5
	$MgSO_4.7H_2O$	14.4		720.0
	Microelements			
(2)	$ZnSO_4.7H_2O$	3.0		3.0
	$MnSO_4.4H_2O$	7.0	1.0	7.0
	H_3BO_3	1.5		1.5
	KI	0.75		0.75
	$CuSO_4.5H_2O$	0.001		0.001
	$Na_2MoO_4.2H_2O$	0.25		0.25
(3)	$FeCl_3$	1.5	1.0	1.5
(4)	IAA	1.0	1.0	1.0
(5)	Vitamins and Amino acids			
	Glycine	3.0		3.0
	Thiamine HCl	0.5	1.0	0.5
	Nicotinic acid	0.5		0.5
	Pyridoxine	0.5		0.5
(6)	Coconut milk		100.0	10%
(7)	Yeast Extract	5		0.5%
(8)	Sucrose	20		2%
(9)	FOR INITIATION OF CALLAS:			
	2,4-D	1.0		1.0
	NAA	1.0		1.0
(10)	Agar	10		1.0%

MODIFIED MURASHIGE AND SKOOG MEDIUM

TISSUES CULTURED ON MEDIUM: PINUS PALUSTRIS

PINUS LAMBERTIANA

PINUS ELLIOTTII

pH 5.7-5.8

	Major Solution	Stock Solution, gm/liter	Stock solution, m/l liter of medium	Final concentration, mg/liter
(1)	NH_4NO_3	16.5		1650.0
	KNO_3	19.0	100.0	1900.0
	$CaCl_2.2H_2O$	4.4		440.0
	$MgSO_4.7H_2O$	3.7		370.0
	KH_2PO_4	1.7		170.0
(2)	Na_2-EDTA	7.45	5	37.3
	$FeSO_4.7H_2O$	5.57		27.8
	Minor Solution			
(3)	HBO_3	6.2		6.2
	$MnSO_4.4H_2O$	22.3		22.3
	$ZnSO_4.4H_2O$	8.6	1.0	8.6
	KI	0.83		0.83
	$Na_2MoO_4.2H_2O$	0.25		0.25
	$CuSO_4.5H_2O$	0.025		0.025
	$CoCl_2.6H_2O$	0.025		0.025
	Vitamins and Amino acids			
(4)	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1	0.1	0.1
	Pyridoxine HCl	0.1		0.1
	Kinetin	0.5		0.5
(5)	2,4-D	5	1.0	5.0
(6)	Inositol	100	1.0	100.0
(7)	Asparagine	100	1.0	100.0
(8)	Ascorbic acid	50	1.0	50.0
(9)	Sucrose	30		3%
(10)	Agar	10		1.0%

WHITE'S 10X MEDIUM

TISSUES CULTURED ON MEDIUM: HABITUATED TOBACCO

PH 5.5-6.0

		Stock Solution, gm/liter	Stock Solution m/l liter of medium	Final concentration, mg/liter
(1)	Macroelements			
	$Ca(NO_3)_2.4H_2O$	6.0		3000.0
	Na_2SO_4	4.0	500.0	2000.0
	KNO_3	1.6		800.0
	KCl	1.3		650.0
	$NaH_2PO_4.H_2O$	0.33		165.0
	$MgSO_4.7H_2O$	14.4		7200.0
	Microelements			
(2)	$ZnSO_4.7H_2O$	3.0		30.0
	$MnSO_4.4H_2O$	7.0		70.0
	H_3BO_3	1.5	10.0	15.0
	KI	0.75		7.5
	$CuSO_4.5H_2O$	0.001		0.01
	$Na_2MoO_4.2H_2O$	0.25		2.5
(3)	$FeCl_3$	1.5	1.0	1.5
	Vitamins and Amino acids			
(4)	Glycine	3.0		3.0
	Thiamine HCl	0.5	1.0	0.5
	Nicotinic acid	0.5		0.5
	Pyridoxine	0.5		0.5
(5)	Sucrose	30		3%
(6)	Agar	10		1%

S. V. MEDIUM (Modified from Murashige and Skoog, 1962)

TISSUES CULTURED ON MEDIUM: SUNFLOWER; CARROT; SOYBEAN, HAPLOPAPPUS

Macronutrients	Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration, mg/liter
(1) NH_4NO_3	82.5	20.0	1650.0
KNO_3	95.0		1900.00
(2) $CaCl_2.2H_2O$	88.0	5.0	440.0
(3) $MgSO_4.7H_2O$	74.0	5.0	370.0
KH_2PO_4	34.0		170.0
(4) Na_2EDTA	7.45	5.0	37.3
$FeSO_4.7H_2O$	5.57		27.8
Micronutrients			
(5) $MnSO_4.H_2O$	4.5		4.5
$ZnSO_4.7H_2O$	1.5		1.5
H_3BO_3	1.5		1.5
$CuSO_4.5H_2O$	0.04	1.0	0.04
$Na_2MoO_4.2H_2O$	0.25		0.25
$CoCl_3.6H_2O$	0.005		0.005
$AlCl_3$	0.003		0.003
$NiCl_2.6H_2O$	0.003		0.003
KI	0.001		0.001
Vitamins and Amino acids			
(6) Nicotinic acid	0.5		0.5
Thiamine HCl	0.1	1.0	0.1
Pyridoxine HCl	0.1		0.1
Glycine	3.0		3.0
(7) NAA	0.175	1.0	0.175
(8) 2,4-D	0.221	1.0	0.221
(9) Yeast Extract	1		0.1%
(10) Coconut Milk		100.0	10%
(11) Ascorbic Acid	50.0		50.0
(12) Sucrose	30		3%
(13) Agar	10		1%